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Pharmacological screen for activities of 12-hydroxyibogamine: a primary metabolite of the indole alkaloid ibogaine

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Abstract The purported efficacy of ibogaine for the treatment of drug dependence may be due in part to an active metabolite. Ibogaine undergoes first pass metabolism and is *O*-demethylated to 12-hydroxyibogamine (12-OH ibogamine). Radioligand binding assays were conducted to identify the potency and selectivity profiles for ibogaine and 12-OH ibogamine. A comparison of 12-OH ibogamine to the primary molecular targets identified previously for ibogaine demonstrates that the metabolite has a binding profile that is similar, but not identical to the parent drug. Both ibogaine and 12-OH ibogamine demonstrated the highest potency values at the cocaine recognition site on the 5-HT transporter. The same rank order (12-OH ibogamine > ibogaine), but lower potencies were observed for the [³H]paroxetine binding sites on the 5-HT transporter. Ibogaine and 12-OH ibogamine were equipotent at vesicular monoamine and dopamine transporters. The metabolite demonstrated higher affinity at the kappa-1 receptor and lower affinity at

the NMDA receptor complex compared to the parent drug. Quantitation of the regional brain levels of ibogaine and 12-OH ibogamine demonstrated micromolar concentrations of both the parent drug and metabolite in rat brain. Drug dependence results from distinct, but inter-related neurochemical adaptations, which underlie tolerance, sensitization and withdrawal. Ibogaine's ability to alter drug-seeking behavior may be due to combined actions of the parent drug and metabolite at key pharmacological targets that modulate the activity of drug reward circuits.

Key words Ibogaine · 12-Hydroxyibogamine · Ligand binding · Neuroreceptors · Neurotransporter · Drug dependence

Introduction

The potential for deriving new psychotherapeutic medications from natural sources has led to renewed interest in rain forest plants for the development of anti-addiction medications. Ibogaine is a rain forest alkaloid found in the root of *Tabernanthe iboga* (Apocynaceae family), a shrub that grows in West Central Africa. Ibogaine is used by native peoples in low doses to combat fatigue, hunger and thirst, and at high doses for its hallucinogenic properties in religious rituals. Ibogaine has been claimed by members of American and European addict self-help groups to promote long-term drug abstinence from addictive substances, including psychostimulants, opiates and alcohol (for review, Popik et al. 1995). The purported efficacy is based on anecdotal reports that after a single dose of ibogaine, symptoms associated with cocaine and opioid withdrawal are eliminated and drug "craving" is inhibited for extended periods of time (Sisko 1993; Lotsof 1995). These findings are supported by preclinical animal studies which demonstrate

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that ibogaine decreased morphine and cocaine self-administration (Glick et al. 1991, 1994; Cappendijk et al. 1994; Sershen et al. 1994) and blocked some of the symptoms of naloxone-precipitated withdrawal (Dzolic et al. 1988; Glick et al. 1992; Cappendijk et al. 1993). Ibogaine is centrally active and at high doses produces adverse effects, including tremors and hallucinations (Popik et al. 1995). The development of ibogaine as an anti-addiction drug has been hindered due to uncertainties over potential neurotoxicity (O'Hearn and Molliver 1993; Touchette 1993). While ibogaine has a variety of CNS effects that are dose-related, the pharmacological targets underlying the physiological and psychological actions of ibogaine are not completely understood.

Ibogaine interacts with multiple targets within the CNS, including dopaminergic, cholinergic, glutamatergic, opioidergic and serotonergic systems (Popik et al. 1995). Radioligand binding surveys of ibogaine in rat brain demonstrated micromolar potencies for the dopamine (DA) transporter (Sershen et al. 1992) kappa-1 and mu opioid receptors, alpha-1 adrenergic receptors and voltage-dependent sodium channels (Deecher et al. 1992; Sweetnam et al. 1995). Ibogaine has low affinity for muscarinic receptor subtypes (Sweetnam et al. 1995) and competitively inhibits [³H]MK-801 binding to the NMDA receptor complex (Popik et al. 1994; Mash et al. 1995a). Ibogaine possesses moderate affinity for putative sigma₂ receptors (Bowen et al. 1995; Mach et al. 1995). Ibogaine administrations cause a 5-HT behavioral syndrome in rats, suggesting an interaction with serotonergic targets (Popik et al. 1995). These multi-site interactions with a diverse grouping of neuropharmacological targets contribute to the wide spectrum of CNS activities. Recent studies have suggested that some of the neurobehavioral and physiological aftereffects of ibogaine may be mediated by a long-lasting metabolite. The principal metabolite of ibogaine has been identified in humans and primates as 12-hydroxyibogamine (12-OH ibogamine; Hearn et al. 1995a; Mash et al. 1995b). In the present study, we used radioligand binding assays to compare the potencies of ibogaine and 12-OH ibogamine at neuroreceptors and neurotransmitters to help define the molecular mechanisms of action that may account for ibogaine's anti-addictive properties.

Materials and methods

Neurological tissue specimens

Human neuropathological tissue specimens were obtained at routine autopsy from accidental death victims (males; age range 20–46 years; autolysis times 13–21 h). Brain tissue specimens were frozen and stored at –70 °C until the day of assay. For binding assays, the region of interest (Table 1) was dissected and membrane homogenates were prepared by homogenizing tissue in ice-cold ligand-specific buffer with a Brinkman polytron (15 s, setting 3).

Membrane homogenates were centrifuged and washed to remove endogenous ligand.

Ligand binding assays

Radioligands were commercially available from NEN/Dupont (Boston, Mass.) or Amersham Corp. (Arlington Heights, Ill.) with the exceptions of [¹²⁵I]iodovinyltetraabenazine and [¹²⁵I]IOXY which were custom synthesized and radiolabeled by Dr. Hank Kung (University of Pennsylvania) and Dr. Richard Rothman (Addiction Research Center/NIDA), respectively. Ibogaine and 12-OH ibogamine were obtained from s.a. Omnicem, Belgium. BIT (2-(*p*-ethoxybenzyl)-1-diethylaminoethyl-5-isothiocyantobenzimidazole-HCl) and FIT (*N*-phenyl-*N*-[1-[2-(4-isothiocyantophenyl)ethyl-4-piperidinyl]propanamide] were synthesized in the Laboratory of Medicinal Chemistry, NIDDK, Bethesda, Md.). All other unlabeled drugs were purchased from Research Biochemicals (Natick, Ma.).

A summary of the radioligands used in competition assays with ibogaine and 12-OH ibogamine is shown in Table 1. All binding assays were conducted as described previously (see Table 1 for references). CHO cells stably transfected with genes for each muscarinic receptor subtype were prepared for ligand binding screens at m1–m5 receptor subtypes as described previously (Ferrari-Dileo et al. 1994). The ability of ibogaine and 12-OH ibogamine to inhibit binding to neuroreceptors or transporters was first assessed at doses of 100 nM and 10 µM. Positive controls were routinely assayed in parallel using specific drugs with known affinities (Table 1). Assay tubes were incubated under the specified conditions and filtered through Whatman 934AH filters on Millipore manifolds. Nonspecific binding was defined as the cpm bound in the presence of a saturating concentration of an established competing ligand. All binding assays demonstrated a total signal of at least 2000 dpm with at least 60–90% specific binding for each radioligand. Ibogaine and 12-OH ibogamine were considered active at the defined receptor site if there was 50% or higher inhibition at a concentration of 10 µM. To determine potency values accurately, full competition curves were obtained at relevant binding sites using 10–15 concentrations of ibogaine or 12-OH ibogamine. Ligand competition data were analyzed using the DRUG program of EBDA/LIGAND (Biosoft, Elsevier).

Quantitative gas chromatography/mass spectrometry (GC/MS)

Using GC/MS, the concentrations of ibogaine and 12-OH ibogamine were determined in brain specimens of rats treated with a single dose of ibogaine (50 mg/kg PO) and killed at the times indicated. Briefly, brain tissue was diluted with 1% NaCl (1:1), homogenized (30 s) and sonicated (5 min). The alkaloids were extracted from the tissue homogenate using a solvent extraction under basic conditions with D₃-ibogaine as an internal standard and derivatization of the metabolite to an ethyl ether as described previously (Hearn et al. 1995a, b). The GC/MS was operated in the full scan electron ionization mode scanning from M/Z 45 to 450 at 1 s/scan. Ibogaine and its principal metabolite were identified by subjecting brain extracts to full scan electron impact GC/MS on Finnegan 4521 (quadrupole) and Finnegan ITS-40 (ion trap) mass spectrometers. Compound identification was based upon comparison of retention times and fragmentation patterns obtained from authentic standards (s.a. Omnicem, Belgium). Ion ratios for the molecular ion of ibogaine (*m/z* – 310), and 12-OH ibogamine ethyl ether (*m/z* – 324) to that of the internal standard *O*-D₃-methyl-ibogaine (*m/z* – 313) were subjected to least squares linear regression versus concentration. The resulting standard curves were linear (*r*² = 0.999) and reproducible. Intraassay coefficients of variation (CV) in brain were 1.1% for ibogaine and 4.0% for 12-OH ibogamine. Limits of detection and

Table 1 Survey of ibogaine and 12-OH ibogamine targets in radioligand binding assays

Binding site	Radioligand	Nonspecific	Tissue	Reference
<i>Adenosinergic</i>				
A ₁ receptor	[³ H]NECA	R-PIA	Caudate	(Bruns et al. 1986)
A ₂ receptor	[³ H]CGS-21680	CGS21680	Caudate	(Jarvis et al. 1989)
<i>Cholinergic</i>				
M ₁ receptor	[³ H]NMS	QNB	CHO-m1	(Ferrari-DiLeo et al. 1994)
M ₂ receptor	[³ H]NMS	QNB	CHO-m2	(Ferrari-DiLeo et al. 1994)
M ₃ receptor	[³ H]NMS	QNB	CHO-m3	(Ferrari-DiLeo et al. 1994)
M ₄ receptor	[³ H]NMS	QNB	CHO-m4	(Ferrari-DiLeo et al. 1994)
M ₅ receptor	[³ H]NMS	QNB	CHO-m5	(Ferrari-DiLeo et al. 1994)
Vesicular transporter	[³ H]Vesamicol	(-) ABV	Caudate	(Kish et al. 1990)
<i>Dopaminergic</i>				
D ₁ receptor	[³ H]SCH23390	(+) Butaclamol	Caudate	(DeKeyser et al. 1989)
D ₂ receptor	[³ H]YM-09151-2	(+) Butaclamol	Caudate	(Jarvie et al. 1987)
	[³ H]Haloperidol	(+) Butaclamol	Caudate	(Whitaker and Seeman 1977)
D ₃ receptor	[³ H]-(+)-7-OH-DPAT	(+) Butaclamol	Nuc. Acc.	(Burris et al. 1994)
DA transporter	[¹²⁵ I]RTI-121	(-) Cocaine	Caudate	(Staley et al. 1995)
	[³ H]Mazindol	(-) Cocaine	Caudate	(Staley et al. 1995)
Vesicular transporter	[¹²⁵ I]-TBZ	Tetrabenazine	Caudate	(Kung et al. 1994)
<i>Glutamatergic</i>				
NMDA receptor complex	[³ H]-(+)-MK801	(+) MK801	Caudate	(Popik et al. 1994)
<i>Noradrenergic</i>				
NE transporter	[³ H]Nisoxetine	Desipramine	Dentate Gyrus	(Tejani-Butt et al. 1990)
<i>Opioidergic</i>				
Kappa ₁	[³ H]U69593	Naloxone	Insular Ctx	(Nock et al. 1988)
Kappa ₂	[¹²⁵ I]-IOXY	Naloxone	Caudate	(Ni et al. 1993)
Sigma ₁	[³ H]-(+)-Pentazocine	(±) Pentazocine	Cerebellum	(Zabetian et al. 1994)
<i>Serotonergic</i>				
5-HT _{1A} receptor	[³ H]-8-OH-DPAT	Serotonin	Hippocampus	(Gozlan et al. 1983)
5-HT ₂ receptor	[³ H]RP 62203	Ketanserin	Frontal Ctx	(Malgouris et al. 1993)
5-HT transporter	[¹²⁵ I]RTI-55	(-) Cocaine	Occipital Ctx	(Staley et al. 1994)
	[³ H]Paroxetine	Paroxetine	Occipital Ctx	(Backstrom et al. 1989)
Vesicular transporter	[¹²⁵ I]-TBZ	Tetrabenazine	Occipital Ctx	(Kung et al. 1994)

quantitation were 5 ng/g for both ibogaine and derivatized 12-OH ibogamine in brain, respectively.

Results

The results of the radioligand binding survey of the potencies of ibogaine and 12-OH ibogamine at various neuroreceptors and neurotransmitters are shown in Table 2. At a 10 μ M dose, both ibogaine and 12-OH ibogamine competed for at least 50% of the total binding to the DA transporter, 5-HT transporter and vesicular monoamine transporter. In addition, ibogaine was active in displacement assays at the cholinergic vesicular transporter and NMDA receptor complex, while 12-OH ibogamine was more potent than ibogaine at the kappa-1 receptor. Both ibogaine and its primary metabolite were nonselective and weak inhibitors of binding to m1-m5 muscarinic receptor subtypes. The results of the present study confirm and extend previous reports that ibogaine and its primary metabolite do not interact with biogenic amine receptors, including dopaminergic (Deecher et al. 1992; Sweetnam et al. 1995) and serotonergic receptor subtypes

(Deecher et al. 1992). Neither ibogaine nor 12-OH ibogamine demonstrated significant binding affinity at the norepinephrine transporter, sigma-1 binding sites or adenosine (A1 and A2) receptor subtypes assayed in human brain.

The significance of micromolar interactions of ibogaine and 12-OH ibogamine with various radioligand binding sites was related to the concentration of parent drug and metabolite in brain. Regional brain levels of ibogaine and 12-OH ibogamine were measured in rat cerebral cortex, striatum, brainstem and cerebellum at 15 min, 1 and 2 h post-drug administration. The results demonstrate that ibogaine is rapidly detected in brain following oral administration (Fig. 1). The metabolite was detected at the earliest time point (15 min), consistent with first pass metabolism of the parent drug (Hearn et al. 1995a). Administration of ibogaine (50 mg/kg PO) in rodents resulted in levels of ibogaine and 12-OH ibogamine ranging from 4 to 17 μ M and 1 to 17 μ M, respectively. It is interesting to note that over the 2-h time period the concentration of 12-OH ibogamine increased 10-fold across all regions examined, while the levels of the parent drug decreased (cortex, brainstem) or stayed the same (striatum, cerebellum). These data provide

Table 2 Empirical screen of the inhibitory potency of ibogaine and 12-OH ibogamine

Inactive (10 μ M) test dose ^a	Active ^b
<i>Ibogaine</i>	
[³ H]SCH 23390	[³ H]MK-801
[³ H]YM01915-2	[³ H]Mazindol
[³ H]-(+)-7-OH-DPAT	[¹²⁵ I]RTI-121
[³ H]Haloperidol	[¹²⁵ I]-TBZ
[³ H]NECA	[¹²⁵ I]RTI-55/Benztropine
[³ H]CGS 21680	[³ H]Paroxetine
[³ H]Nisoxetine	[³ H]Vesamicol
[³ H]-8-OH-DPAT	[³ H]NMS-CHO-m1
[³ H]RP62203	[³ H]NMS-CHO-m2
[³ H]NMS-CHO-m5	[³ H]NMS-CHO-m3
[³ H]-(+)-Pentazocine	[³ H]NMS-CHO-m4
[³ H]U69593	
[¹²⁵ I]IOXY	
<i>12-OH Ibogamine</i>	
[³ H]SCH 23390	[³ H]Mazindol
[³ H]YM 01915-2	[¹²⁵ I]RTI-121
[³ H]-(+)-7-OH-DPAT	[¹²⁵ I]-TBZ
[³ H]-8-OH-DPAT	[¹²⁵ I]RTI-55/Benztropine
[³ H]RP62203	[³ H]Paroxetine
[³ H]Vesamicol	[³ H]U69593
[³ H]NECA	[³ H]NMS-CHO-m1
[³ H]Haloperidol	[³ H]NMS-CHO-m2
[³ H]-(+)-Pentazocine	[³ H]NMS-CHO-m3
[³ H]CGS21680	[³ H]NMS-CHO-m4
[³ H]Nisoxetine	[³ H]NMS-CHO-m5
[³ H]MK-801	
[¹²⁵ I]IOXY	

^aInactive - Ibogaine or 12-OH ibogamine competed for binding with an IC₅₀ value greater than 10 μ M

^bActive - Ibogaine or 12-OH ibogamine inhibited binding with an IC₅₀ value equal to or less than 10 μ M

tion sites on the 5-HT transporter (IC₅₀ = 40.7 $\pm$ 11.6 nM). Interestingly, 12-OH ibogamine competed for [³H]paroxetine binding to the 5-HT transporter with 20-fold lower potency (IC₅₀ = 0.9 $\pm$ 0.06 μ M) than at RTI-55 binding sites. In keeping with the demonstrated rank order of potency, 12-OH ibogamine was 10-fold more potent than ibogaine at displacing both [¹²⁵I]RTI-55 and [³H]paroxetine binding (Table 3). The metabolite also was more potent than the parent drug at the kappa-1 receptor and displaced binding in the low micromolar range. Ibogaine displayed 4- and 6-fold higher potencies than the metabolite at kappa-2 receptors and at MK-801 sites on the NMDA receptor complex, respectively. Ibogaine and 12-OH ibogamine were equipotent at the DA transporter and vesicular monoamine transporter and displaced binding in the low micromolar range (Fig. 2, Table 3).

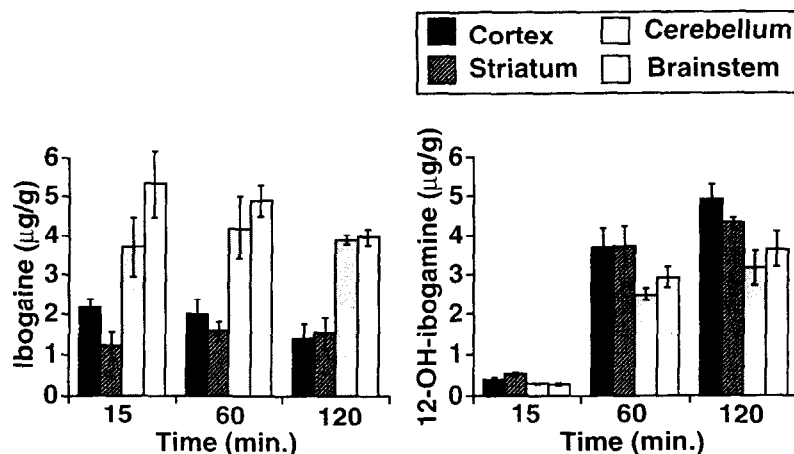
Discussion

The present study provides additional insights into the ligand binding profiles for ibogaine and its primary metabolite that may be relevant for understanding the putative anti-addictive properties. Comparison of the in vitro binding profiles for ibogaine and 12-OH ibogamine demonstrates that they interact with neurotransmitters and subclasses of neurotransmitter receptors, which are associated with the reinforcing actions of cocaine and opiates. Ibogaine and 12-OH ibogamine are active at specific pharmacological targets that have been linked to the phenomena of sensitization and tolerance. While micromolar potencies of ibogaine and its desmethyl metabolite may be considered ancillary for defining primary sites and mechanisms of action (Sweetnam et al. 1995), our pharmacokinetic studies demonstrate that both ibogaine and 12-OH ibogamine are found in rat brain following oral administrations (50 mg/kg) at levels ranging from 1 to 17 μ M. The pharmacological relevance of micromolar brain

further evidence that micromolar activities of ibogaine and the *O*-demethylated metabolite are relevant for defining binding site activities.

Ibogaine and 12-OH ibogamine exhibited distinct neurotransmitter and neuroreceptor binding profiles (Fig. 2). The highest potency value observed in this activity screen was for the inhibition of [¹²⁵I]RTI-55 binding by 12-OH ibogamine at cocaine recogni-

Fig. 1 Regional brain levels of ibogaine (*left*) and 12-OH ibogamine (*right*). Regional samples of rat cerebral cortex, striatum, cerebellum and brainstem were assayed at the indicated times post-administration of ibogaine (50 mg/kg PO). Data represent the average values from individual animals (*n* = 4) assayed in duplicate



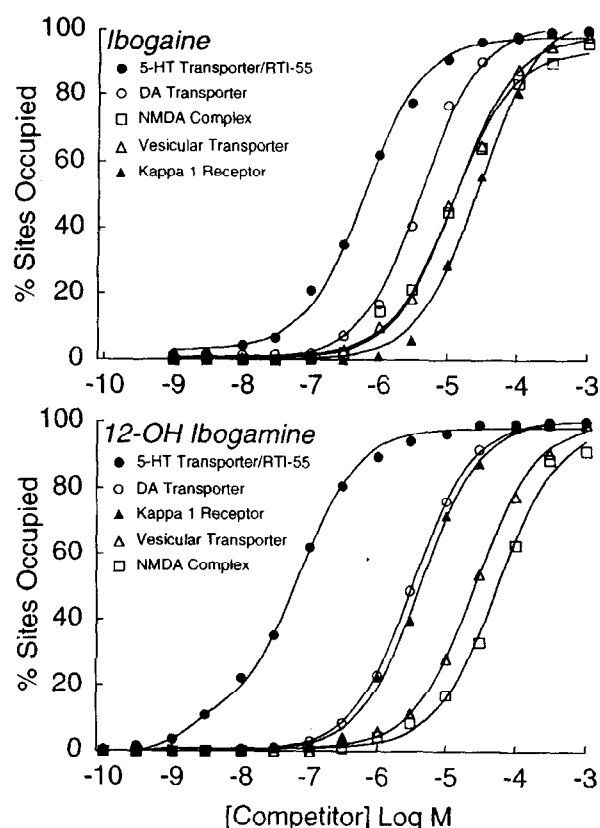


Fig. 2 Potency of ibogaine and 12-OH ibogamine for binding to CNS targets. Various concentrations of ibogaine (*upper panel*) and 12-OH ibogamine (*bottom panel*) were incubated in the presence of [125 I]RTI-55 (10 pM)/1 μ M benztrapine, [125 I]RTI-121 (50 pM), [3 H]MK-801 (2 nM), [125 I]TBZ (1 nM) and [3 H]U69593 (1 nM) with membrane homogenates from the human brain. The data shown are the mean $\pm$ SE of at least three independent experiments. Potency values were obtained using DRUG (EBDA/LIGAND)

concentrations of ibogaine and 12-OH ibogamine is supported by previous studies which have demonstrated that doses of ibogaine within the range of 30–80 mg/kg are active in behavioral studies designed to assess reinforcing effects, tolerance and withdrawal symptoms

Table 3 Inhibitory potency of ibogaine and 12-OH ibogamine. The values shown represent the mean $\pm$ SE of the IC_{50} value (μ M) from 3–4 independent experiments each performed in triplicate

Binding target (Radioligand)	Ibogaine		12-OH Ibogamine	
	IC_{50} (μ M)	nH	IC_{50} (μ M)	nH
<i>Dopaminergic</i>				
DA transporter (RTI-55)	4.11 $\pm$ 0.45	1.10	3.35 $\pm$ 0.50	1.01
Vesicular transporter (TBZ)	14.63 $\pm$ 4.47	0.98	29.45 $\pm$ 9.13	1.05
<i>Glutamatergic</i>				
NMDA receptor (MK-801)	5.20 $\pm$ 0.24	0.90	31.41 $\pm$ 5.43	1.05
<i>Opioidergic</i>				
Kappa $_1$ (U69593)	25.00 $\pm$ 0.57	1.09	4.24 $\pm$ 0.28	1.05
Kappa $_2$ (IOXY)	23.80 $\pm$ 7.10	0.99	92.30 $\pm$ 9.21	1.03
<i>Serotonergic</i>				
5-HT transporter (RTI-55)	0.59 $\pm$ 0.09	0.80	0.04 $\pm$ 0.01	0.76
(paroxetine)	9.30 $\pm$ 1.70	1.15	0.90 $\pm$ 0.06	1.10
Vesicular transporter (TBZ)	2.23 $\pm$ 0.22	0.75	4.99 $\pm$ 0.48	0.98

associated with psychomotor stimulants and opiates. Ibogaine has been shown to reduce signs of morphine withdrawal (Glick et al. 1992), antagonize cocaine-induced locomotor stimulation (Sershen et al. 1992) and inhibit cocaine (Cappendijk and Dzoljic 1993; Glick et al. 1994) and morphine self-administration (Glick et al. 1994).

At present, the metabolism and intracerebral disposition of ibogaine in the brain is not completely known. Both the parent drug and metabolite have high heptane/phosphate buffer partition coefficients (Zetler et al. 1972), indicating their ability to penetrate the blood-brain barrier and consistent with the rapid entry of ibogaine into the brain shown here. Partitioning of the parent drug into brain lipid may serve as a slow release storage "depot". Sequestration of iboga alkaloids into lipophilic compartments in brain, may result in lower concentrations of the parent drug and metabolite in the extracellular fluid. The more polar nature of 12-OH ibogamine suggests that there may actually be higher extracellular fluid concentrations of the metabolite as compared to ibogaine. If ibogaine is *O*-demethylated to 12-OH ibogamine in brain, then it is reasonable to conclude that the slow elimination of a CNS trapped polar metabolite may contribute to some of the reported aftereffects of single dose administrations of ibogaine in humans (Mash et al. 1995b). However, further studies are needed to estimate precisely the intracerebral distribution of ibogaine and 12-OH ibogamine and their relative concentrations in extracellular fluid as compared to brain extracts.

The metabolite 12-OH ibogamine inhibited binding of the cocaine congener [125 I]RTI-55 to the 5-HT transporter with the highest potency reported to date. Interestingly, ibogaine and 12-OH ibogamine exhibited 15- to 20-fold lower potency for inhibition of paroxetine binding to the 5-HT transporter. The disparity in the potency values for binding to the 5-HT transporter suggests that 12-OH ibogamine and ibogaine may recognize a binding domain on the transporter that is preferred by cocaine-like, but not structurally dissimilar

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transport inhibitors. Previous studies have shown that there are multiple cocaine-like recognition sites associated with the 5-HT transporter (Rothman et al. 1994; Staley et al. 1994). Interestingly, paroxetine has been shown to label two RTI-55 recognition sites on the 5-HT transporter with distinct affinities (Rothman et al. 1994). These observations provide additional evidence for the non-identity of binding sites associated with the 5-HT transporter and suggest that ibogaine and its desmethyl metabolite may be useful probes for dissecting their functional relevance.

In keeping with its potency at the 5-HT transporter, previous studies have shown that 12-OH ibogamine elevates extracellular levels of 5-HT in microdialysis assays following intravenous injections in rats (Mash et al. 1995b). Preliminary studies have suggested that drugs (fluoxetine, sertraline) that enhance 5-HT neurotransmission are indicated for the treatment of heroin and cocaine dependence (Batki et al. 1994; Kleber 1995; Walsh et al. 1994). Chronic drug use is associated with a 5-HT deficit form of 5-HT dysregulation, causing depressed mood and alterations in affect and cognition (Baumann et al. 1993, 1995; Levy et al. 1994; Parsons et al. 1995). Improvements in mood and cognition have been associated with the alleviation of drug "craving", which is a predictor of drug relapse. Mood measures are also important indicators of the effect of treatment for cocaine abuse, and some aspects of cravings intersect with depression and anxiety (Covi et al. 1995). These observations suggest that the interaction of a longer acting metabolite with the 5-HT transporter may be relevant for explaining the reported ability of ibogaine to rapidly alleviate dysphoria and improve mood and cognition in abstinent-drug abusers.

While ibogaine and 12-OH ibogamine displayed high affinity for the 5-HT transporter, both the parent and metabolite failed to demonstrate significant potency for binding to the 5-HT_{1A} and 5-HT₂ receptors assayed in human brain. These results conflict with a previous report, which demonstrated potencies in the low micromolar range for ibogaine binding to the 5-HT₂ receptor subtype (Sweetnam et al. 1995). In this study, [³H] ketanserin was used to label the 5-HT₂ receptor subtype. It is known that this ligand binds with nanomolar affinity to the vesicular monoamine transporter (Darchen et al. 1988). This inconsistency between studies may reflect species differences or the overlapping affinities of [³H] ketanserin for the 5-HT₂ receptor and vesicular monoamine transporter. The lack of affinity of ibogaine for 5-HT₂ receptors shown here may indicate that the hallucinogenic activities of the drug may be mediated by either a different population of 5-HT receptor or that ibogaine does not fit the binding site profile of classical hallucinogens (Glennon 1990).

The rewarding effects of abused drugs are mediated by activation of the mesolimbic DA system (reviewed

by Di Chiara 1995). In particular, the DA transporter is a primary substrate for the reinforcing potential of psychostimulants. Ibogaine recognizes the cocaine binding site on the DA transporter and inhibits DA uptake in rat brain with a potency value corresponding to its affinity for the cocaine binding site (M. Baumann, personal communication). These findings suggest that ibogaine and 12-OH ibogamine may interact with the DA transporter in a similar manner to cocaine, but with 10-fold lower potency (Staley et al. 1994). Ibogaine and its primary metabolite may function as "cocaine partial agonists" at the DA transporter by blocking the access of cocaine and decreasing the rapid elevation of DA mediating the rewarding effects of cocaine. This explanation may account for ibogaine's ability to decrease cocaine place preference and inhibit cocaine self-administration (Cappendijk et al. 1994; Glick et al. 1994; Sershen et al. 1994). Ibogaine may affect DA transmission also by promoting a redistribution of DA synaptosomal pools in addition to its blockade of DA transporter function. Ibogaine has been shown to increase DA efflux from the cytoplasmic pool by reversal of the DA transporter (Harsing et al. 1994). Since ibogaine displays moderate affinity at the vesicular monoamine transporter, it may regulate the distribution of DA between vesicular and cytoplasmic pools. If ibogaine blocks vesicular storage of DA, the higher concentrations of cytoplasmic DA would lead to reversal of the DA transporter. In keeping with this "redistribution" hypothesis, ibogaine pretreatment significantly reduced the cocaine-induced rise in DA levels in the nucleus accumbens measured by in vivo voltametry (Broderick et al. 1994).

Mesolimbic DA neurotransmission is known to be modulated by tonic activation of the mu and kappa opioid receptors within the limbic reward sectors of the striatum. According to the model by Spanagel and Shippenberg (1992), opioids tonically regulate mesolimbic DAergic transmission through two opposing mechanisms that regulate DA release via distinct actions at cell body and terminal fields. Activation of mu receptors increase, whereas kappa agonists decrease DA release in the nucleus accumbens. Ibogaine and 12-OH ibogamine have demonstrated micromolar affinities for mu and kappa opioid receptors (Pearl et al. 1995), suggesting an additional mechanism for regulating the DAergic reward pathway. We have confirmed that ibogaine and 12-OH ibogamine have affinities in the low micromolar range at kappa receptor subtypes in agreement with previous reports (Deecher et al. 1992; Reid et al. 1994; Pearl et al. 1995; Sweetnam et al. 1995). In the present study, the *O*-demethylated metabolite displayed the highest potency at kappa-1 receptor sites assayed in human brain membranes. The role of specific kappa receptor subtypes within limbic reward pathways of the human brain are at present unknown. The selective kappa-1

agonist U-69593 attenuates cocaine-induced behavioral sensitization (Heidbreder et al. 1993), while the non-selective kappa antagonist nor-binaltorphimine enhances morphine-induced sensitization (Spanagel et al. 1992; Spanagel and Shippenberg 1993). Ibogaine and 12-OH ibogamine may regulate DA release via their interactions with kappa receptors (Reid et al. 1994). The actions of the parent drug and metabolite at kappa receptors may be important for understanding the anti-craving aftereffects of ibogaine. While kappa agonists are markedly dysphoric, the additional and potent actions of 12-OH ibogamine at the 5-HT transporter may significantly elevate mood, and thereby limit the potential adverse effects of kappa receptor activation.

The ability of ibogaine to inhibit [^3H]-MK801 binding to the NMDA receptor complex (Popik et al. 1994; Mash et al. 1995b; Sweetnam et al. 1995) may be of relevance for understanding the anti-addictive actions of ibogaine. MK-801 has been reported to block sensitization (reverse tolerance) to the behavioral activating effects of cocaine and amphetamine (Karler et al. 1989; Pudiak and Bozarth 1993). The administration of MK-801 attenuates both the development of tolerance to the analgesic effect of morphine and morphine dependence (Trujillo and Akil 1991, 1995). On the basis of these converging lines of evidence, Skolnick and coworkers suggest that ibogaine's ability to modify drug-seeking behavior results primarily from their blockade of NMDA receptor-coupled cation channels (Popik et al. 1994, 1995). Previously, we have shown that 12-OH ibogamine is less potent than ibogaine at inhibiting [^3H] MK-801 binding in assays of human brain striatum and cerebellum (Mash et al. 1995b). The effects of MK-801 on sensitization and withdrawal described above are compatible with the idea that some of the anti-addictive properties of ibogaine may result from an interaction with NMDA receptor-coupled cation channels. Since 12-OH ibogamine inhibits [^3H]MK-801 binding with a lower potency as compared to ibogaine, it is unlikely that the channel activity of 12-OH ibogamine is additive to ibogaine. This observation may indicate that some of the acute effects of ibogaine administration, but not the extended aftereffects, may be linked to its activities at MK-801 binding sites. It has been suggested also that ibogaine's interaction with NMDA receptor-coupled cation channels may contribute to the adverse effects of the drug, including the psychotropic (PCP-like actions) and high dose neurotoxic changes in cerebellar Purkinje cells (Sweetnam et al. 1995). Further studies are needed to address the functional significance of NMDA-receptor coupled channel blockade to ibogaine's purported anti-addiction properties.

In the present study, radioligand binding assays were conducted with 12-OH ibogamine to identify the molecular mechanism(s) accounting for ibogaine's putative long-lasting anti-addictive properties. The results

demonstrate that ibogaine and its primary metabolite have pharmacologic sites of action associated with biogenic amine systems, opioidergic and glutamatergic synapses. Dependence on psychostimulants and opiates results from distinct, but inter-related processes which include tolerance, sensitization and withdrawal (Nestler and Hyman 1993, Nestler 1994). The neurochemical substrates underlying drug tolerance and reverse tolerance (sensitization), as well as the expression of withdrawal symptoms are likely to be unique and separate processes occurring within discrete cellular locations and affecting distinct molecular targets. The multi-target CNS actions of ibogaine and its primary metabolite suggest that simultaneous modulation of two or more neural mechanisms may be a more effective approach for designing anti-addiction agents.

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References

- Backstrom I, Bergstrom M, Marcusson (1989) High affinity [^3H]paroxetine binding to serotonin uptake sites in human brain tissue. *J Brain Res* 486:261-268
- Batki SL, Manfredi LB, Peyton J, Jones RT (1994) Fluoxetine for cocaine dependence in methadone maintenance: quantitative plasma and urine cocaine/benzoylcegonine concentrations. *J Clin Psychopharmacol* 13:243-250
- Baumann MH, Brockington AM, Rothman RB (1993) Withdrawal from chronic cocaine enhances behavioral sensitivity to the 5-HT $_2$ /1c agonist DOI. *Biol Psychiatry* 34:576-577
- Baumann MH, Becketts KM, Rothman RB (1995) Evidence for alterations in presynaptic serotonergic function during withdrawal from cocaine in rats. *Eur J Pharmacol* 282:87-93
- Bowen WD, Vilner BJ, Williams W, Bertha CM, Kuehne ME, Jacobson AE (1995) Ibogaine and its congeners are sigma $_2$ receptor-selective ligands with moderate affinity. *Eur J Pharmacol* 279:R1-R3
- Broderick PA, Phelan FT, Eng F, Wechsler RT (1994) Ibogaine modulates cocaine responses which are altered due to environmental habituation: in vivo microvoltametric and behavioral studies. *Pharmacol Biochem Behav* 49:711-728
- Bruns RF, Lu GH, Pugsley TA (1986) Characterization of the A $_2$ adenosine receptor labeled by [^3H]NECA in rat striatal membranes. *Mol Pharmacol* 29:331-346
- Burris KD, Filtz TM, Chumpradit S, Kung MP, Foulon C, Hensler JG, Kung HF, Molinoff PB (1994) Characterization of [^{125}I](R)-*trans*-7-hydroxy-2-[N-propyl-N-(3'-iodo-2'-propenyl)amino] tetralin binding to dopamine D $_3$ receptors in rat olfactory tubercle. *J Pharmacol Exp Ther* 268:935-942
- Cappendijk SLT, Dzoljic MR (1994) Inhibitory effects of ibogaine on cocaine self-administration in rats. *Eur J Pharmacol* 241:261-265
- Cappendijk SL, De Vries R, Dzoljic MR (1993) Excitatory amino acid receptor antagonists and naloxone-precipitated withdrawal syndrome in morphine-dependent mice. *Eur Neuropsychopharmacol* 3:111-116
- Covi L, Hess JM, Kreiter NA, Haertzen CA (1995) Effects of combined fluoxetine and counseling in the outpatient treatment of cocaine abusers. *Am J Drug Alcohol Abuse* 21:327-344

- Darchen F, Scherman D, Laduron PM, Henry J-P (1988) Ketanserin binds to the monoamine transporter of chromaffin granules and of synaptic vesicles. *Mol Pharmacol* 33: 672-677
- Deecher DX, Teitler M, Soderlund DM, Bornmann WG, Kuehne ME, Glick SD (1992) Mechanisms of action of ibogaine and harmaline congeners based on radioligand binding studies. *Brain Res* 571: 242-247
- DeKeyser J, Walraevens H, Ebinger G, Vauquelin G (1989) In human brain two subtypes of D₁ receptors can be distinguished on the basis of differences in guanine nucleotide effect on agonist binding. *J Neurochem* 53:1096-1102
- Di Chiara G (1995) Psychobiology of the role of dopamine in drug-abuse and addiction. *Neurosci Res Commun* 17:133-143
- Dzolic ED, Kaplan CD, Dzolic MR (1988) Effect of ibogaine on naloxone-precipitated withdrawal syndrome in chronic morphine dependent rats. *Arch Int Pharmacodyn Ther* 294:64-70
- Ferrari-Dileo G, Waelbroeck M, Mash DC, Flynn DD (1994) A novel strategy for the selective labeling and localization of the M4 (m4) muscarinic receptor subtype. *Mol Pharmacol* 46: 1028-1035
- Glennon RA (1990) Do classical hallucinogens act as 5HT₂ agonists or antagonists? *Neuropsychopharmacology* 3:509-517
- Glick SD, Rossman K, Steindorf S, Maisonneuve IM, Carlson J N (1991) Effects and aftereffects of ibogaine on morphine self-administration in rats. *Eur J Pharmacol* 195:341-345
- Glick SD, Rossman K, Rao NC, Maisonneuve IM, Carlson JN (1992) Effects of ibogaine on acute signs of morphine withdrawal in rats: independence from tremor. *Neuropharmacology* 31:497-500
- Glick SD, Kuehne ME, Raucci J, Wilson TE, Larson D, Keller RW, Carlson NJ (1994) Effects of *iboga* alkaloids on morphine and cocaine self-administration in rats: relationship to tremorigenic effects and to effects on dopamine release in nucleus accumbens and striatum. *Brain Res* 657:14-22
- Gozlan H, El Mestikaway S, Pichat L, Glowinski J, Hamon M (1983) Identification of presynaptic serotonin autoreceptors using a new ligand: ³H-PAT. *Nature* 305:140-142
- Harsing LG, Sershen H, Lajtha A (1994) Evidence that ibogaine releases dopamine from the cytoplasmic pool in isolated mouse striatum. *J Neural Transm* 96:215-225
- Hearn WL, Mash DC, Pablo J, Hime G, Sambol NC, Doepel FM (1995a) Pharmacokinetics of Ibogaine: analytical methods, animal-human comparisons, and the identification of a primary metabolite. In: Spiehler, V (ed) proceedings of the TIAFT-SOFT Joint Congress. Omnipress, Ann Arbor, Mich., pp 325-334
- Hearn WL, Pablo J, Hime GW, Mash DC (1995b) Identification and quantification of ibogaine and an *O*-demethylated metabolite in brain and biological fluids using gas chromatography-mass spectrometry. *J Anal Toxicol* 19:427-434
- Heidbreder CA, Goldberg SR, Shippenberg TS (1993) The kappa opioid receptor agonist U-69593 attenuates cocaine-induced behavioral sensitization in the rat. *Brain Res* 616:335-338
- Jarvie KR, Niznik HB, Seeman P (1987) Dopamine D-2 receptors in canine brain: ionic effects on [³H]neuroleptic binding. *Eur J Pharmacol* 144:163-171
- Jarvis MF, Schulz R, Hutchison AJ, Do UH, Sills MA, Williams M (1989) [³H]CGS 21680, a selective A₂ adenosine receptor agonist directly labels A₂ receptors in rat brain. *J Pharmacol Exp Ther* 251:888-893
- Karler R, Calder LD, Chaudhry IA, Turkanis SA (1989) Blockade of 'reverse tolerance' to cocaine and amphetamine by MK-801. *Life Sci* 45:500-606
- Kish SJ, Distefano LM, Dozie S, Robitaille Y, Rajput A, Deck JH, Hornykiewicz O (1990) [³H]Vesamicol binding in human brain cholinergic deficiency disorders. *Neurosci Lett* 117:347-352
- Kleber HD (1995) Pharmacotherapy, current and potential, for treatment of cocaine dependence. *Clin Neuropharmacol* 18 [Suppl.1]:S96-S109
- Kung MP, Canney DJ, Frederick D, Zhuang Z, Billings JJ, Kung HF (1994) Binding of [¹²⁵I]-iodovinyltetraabenazine to CNS vesicular monoamine transport sites. *Synapse* 18:225-232
- Levy AD, Baumann MH, Van de Kar LD (1994) Monoaminergic regulation of neuroendocrine function and its modification by cocaine. *Frontiers Neuroendocrinol* 15:85-156
- Lots of HS (1995) Ibogaine in the treatment of chemical dependency disorders: clinical perspectives. *Multidisciplinary Association for Psychedelic Studies* 5:16-27
- Mach RH, Smith CR, Childers SR (1995) Ibogaine possesses a selective affinity for sigma₂ receptors. *Life Sci* 57:PL57-62
- Malgoures C, Flamand F, Doble A (1993) Autoradiographic studies of RP 62203, a potent 5-HT₂ receptor antagonist. Pharmacological characterization of [³H]RP62203 binding in the rat brain. *Eur J Pharmacol* 233:36-45
- Mash DC, Staley JK, Pablo JP, Holohean AM, Hackman JC, Davidoff RA (1995a) Properties of ibogaine and its principal metabolite (12-hydroxyibogamine) at the MK-801 binding site of the NMDA complex. *Neurosci Lett* 192:53-56
- Mash DC, Staley JK, Baumann MH, Rothman RB, Hearn WL (1995b) Identification of a primary metabolite of ibogaine that targets serotonin transporters and elevates serotonin. *Life Sci* 57:PL45-50
- Nestler EJ (1994) Molecular neurobiology of drug addiction. *Neuropsychopharmacology* 11:77-87
- Nestler EJ, Hyman S (1993) Molecular foundations of psychiatry. American Psychiatric Press, Washington, DC
- Ni Q, Xu H, Partilla JS, de Costa BR, Rice KC, Rothman RB (1993) Selective labeling of kappa 2 opioid receptors in rat brain by [¹²⁵I]IOXY: interaction of opioid peptides and other drugs with multiple kappa 2a binding sites. *Peptides* 14:1279-1293
- Nock B, Rajpara A, O'Connor LH, Cicero TJ (1988) Autoradiography of [³H]U69593 binding sites in rat brain: evidence for kappa opioid receptor subtypes. *Eur J Pharmacol* 154:27-34
- O'Hearn E, Molliver ME (1993) Degeneration of Purkinje cells in parasagittal zones of the cerebellar vermis after treatment with ibogaine or harmaline. *Neuroscience* 55:303-310
- Parsons LH, Koob GF, Weiss F (1995) Serotonin dysfunction in nucleus accumbens of rats during withdrawal after unlimited access to intravenous cocaine. *J Pharmacol Exp Ther* 274: 1182-1191
- Pearl SM, Herrick-Davis K, Teitler M, Glick SD (1995) Radioligand-binding study of noribogaine, a likely metabolite of ibogaine. *Brain Res* 675:342-344
- Popik P, Leyer RT, Skolnick P (1994) The putative anti-addictive drug ibogaine is a competitive inhibitor of [³H]MK-801 binding to the NMDA receptor complex. *Psychopharmacology* 114:672-674
- Popik P, Leyer RT, Sholnick P (1995) 100 Years of ibogaine: neurochemical and pharmacological actions of a putative anti-addictive drug. *Pharmacol Rev* 47:235-253
- Pudiak CM, Bozarth MA (1993) L-NAME and MK-801 attenuate sensitization to the locomotor-stimulating effect of cocaine. *Life Sci* 53:1517-1524
- Reid MS, Souza KH, Broderick P, Berger P (1994) Evidence that ibogaine modulates dopamine via a kappa receptor mechanism. *NIDA Res Monogr* 153:392
- Rothman RB, Cadet JL, Akunne HC, Silverthorn ML, Baumann MH, Carroll FI, Rice KC, de Costa BR, Partilla JS, Wang JB, Uhl G, Glowa JR, Dersch CM (1994) Studies of the biogenic amine transporters. IV. Demonstration of a multiplicity of binding sites in rat caudate membranes for the cocaine analog [¹²⁵I]RTI-55. *J Pharmacol Exp Ther* 270:296-309
- Sershen H, Hashim A, Harsing L, Lajtha A (1992) Ibogaine antagonizes cocaine-induced locomotor stimulation in mice. *Life Sci* 50:1079-1086
- Sershen H, Hashim A, Lajtha A (1994) Ibogaine reduces preference for cocaine consumption in C57BL/6By mice. *Pharmacol Biochem Behav* 47:13-19

- Sisko B (1993) Interrupting drug dependency with ibogaine: a summary of four case histories. *Multidisciplinary Association for Psychedelic Studies IV*: 15-24
- Spanagel R, Shippenberg TS (1993) Modulation of morphine-induced sensitization by endogenous κ -opioid systems *Neurosci Lett* 153:232-236
- Spanagel R, Herz A, Shippenberg TS (1992) Opposing tonically active endogenous opioid systems modulate the mesolimbic dopaminergic pathway. *Proc Natl Acad Sci USA* 89: 2046-2050
- Staley JK, Basile M, Flynn DD, Mash DC (1994) Visualizing dopamine and serotonin transporters in the human brain with the potent cocaine analogue [125 I]RTI-55: in vitro binding and autoradiographic characterization. *J Neurochem* 62:540-556
- Staley JK, Boja JW, Carroll FI, Seltzman H, Wyrick CD, Lewin AH, Abraham P, Mash DC (1995) Mapping the dopamine transporter in human brain with the novel selective cocaine analog [125 I]RTI-121. *Synapse* 21:364-372
- Sweetnam PM, Lancaster J, Snowman A, Collins JL, Perschke S, Bauer C, Ferkany J (1995) Receptor binding profile suggests multiple mechanisms of action are responsible for ibogaine's putative anti-addictive activity. *Psychopharmacology* 118: 369-376
- Tejani-Butt S, Brunswick DJ, Frazer A (1990) [3 H]Nisoxetine: a new radioligand for norepinephrine uptake sites in brain. *Eur J Pharmacol* 191:239-243
- Touchette N (1993) Ibogaine neurotoxicity raises new questions in addiction research. *NIH Res* 5:50-55
- Trujillo KA, Akil H (1991) Inhibition of morphine tolerance and dependence by the NMDA receptor antagonist MK-801. *Science* 251:85-87
- Trujillo KA, Akil H (1995) Excitatory amino acids and drugs of abuse: a role for *N*-methyl-D-aspartate receptors in drug tolerance, sensitization and physical dependence. *Drug Alcohol Depend* 38:139-154
- Walsh SL, Preston KL, Sullivan JT, Fromme R, Bigelow GE (1994) Fluoxetine alters the effects of intravenous cocaine in humans. *J Clin Psychopharmacol* 14:396-407
- Whitaker PM, Seeman P (1977) Hallucinogen binding to dopamine/neuroleptic receptors. *J Pharm Pharmacol* 29: 506-507
- Zabetian CP, Staley JK, Flynn DD, Mash DC (1994) [3 H]-(+)-Pentazocine binding to sigma recognition sites in human cerebellum. *Life Sci* 55:389-395
- Zetler G, Singbartl G, Schlosser L (1972) Cerebral pharmacokinetics of tremor-producing harmala and iboga alkaloids. *Pharmacology* 7:237-248

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